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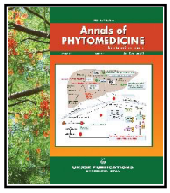

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Formulation and evaluation of herbal gel for the management of acne

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Abstract

The present research involved developing a herbal gel with plant extracts that have anti-keratolytic, anti-inflammatory, antimicrobial and antioxidant properties to treat acne's morbidities. The hydroalcoholic extract (70%v/v) of leaves of *Cassia tora*, flowers of *Cassia auriculata*, roots of *Hemidesmus indicus*, fruits of *Terminalia chebula* and roots of *Glycyrrhiza glabra* along with pharmaceutically acceptable excipients were incorporated in the formulation. The hydroalcoholic extract of selected plants were tested for the presence of metabolites. The prepared herbal gel was subjected to physical evaluation and screened for antiacne activity against the causative organisms *Propionibacterium acne* and *Staphylococcus epidermidis* by well diffusion method. The herbal showed minimum inhibitory concentration of about 63 µg/ml against *S. epidermidis* and 125 µg/ml against *P. acne*. The results of antiacne study demonstrated that the prepared herbal gel exhibits a potent antibacterial activity against the causative microorganisms of acne *P. acne* and *S. epidermidis*, with maximum inhibition comparable with standard. Thus, the significant antibacterial activity of herbal gel may be due to their secondary metabolites/phytochemicals. The physicochemical parameters of the formulation were also optimal with no signs of irritation. Hence, the study can be concluded that among various available topical herbal formulations for acne in the market, the present work proposes making use of these plant extracts for getting better results free from side effects for the benefit of mankind.

1. Introduction

The most prevalent skin condition, acne vulgaris, affects around 80% of people between the ages of 11 and 35. Approximately two million teenagers see doctors annually, with 0.2 million of those visits coming from adults over the age of thirty-five (Dessinioti and Katsambas, 2010; Krautheim and Gollnick, 2004). Acne is thought to cause equal, or occasionally more, psychological, social, and emotional impairment than conditions including diabetes, rheumatoid arthritis, epilepsy, and asthma. Every race and ethnicity is equally affected by acne. Acne is a condition brought on by the obstruction of sebaceous follicles, which are mainly found on the face and trunk and produce excessive amounts of sebum. It shows symptoms such as patches of red, scaly skin (seborrhea), comedones (black and white pimples), nodules (big papules), and occasionally pimples (scarring). Typically, severe acne is inflammatory, although it can also be non-inflammatory. The two bacteria that cause acne are *Propionibacterium acnes* and *Staphylococcus epidermidis* (Bialecka *et al*., 2005). While acne does not present a significant risk to overall health, it is among the most socially upsetting conditions, particularly for young people.

Systemic antibiotics, cymedolytics, exfoliants, and oral and topical bacteriostatic are among the current treatments for acne. The ideal topical formulations for treating acne would have little to no oil and not leave an oily film on the face, which would exacerbate the issue (Lavers, 2014). Major research efforts have been focused on creating an acne control composition that can effectively combat the four stages of acne; namely, sebum generation, hyperkeratinization, *Propionibacterium acne* infection, and inflammation, in order to treat the afore mentioned difficulties associated with acne vulgaris (Olutunmbi *et al.*, 2008). Seborrhea (excess grease), inflammatory lesions (papules and pustules), non-inflammatory lesions (open and closed comedones), and varying degrees of scarring from cyst formation are the clinical symptoms of acne. The several pathological elements that lead to the development of acne have been proven to make combination products more successful in treating acne than monotherapy (Krakowski *et al*., 2008). The target for acne therapy is the four well-known pathogenic factors responsible for this disease state such as antioxidant, anti- inflammatory, decreasing excess sebum production, altering altered follicular keratinisation and decreasing *P. acne* population (Fox *et al*., 2016; Seidler and Kimball, 2010). To combat the morbidities of acne, the present research work was done with the formulation of herbal gel containing plant extracts possessing antikeratolytic, antimicrobial, antioxidant and anti-inflammatory activity. The present invention is an outcome of research to identify an herbal remedy possess anti-inflammatory, antikeratolytic, sebum control and antibacterial agent and methods of developing the same as cosmeceutical product for topical application for control and treatment of *P. acne*. The plants selected for the proposed study were leaves of *C. tora,* flowers of *C. auriculata,* roots of *H. indicus*, fruits *of T. chebula* and roots of *G. Glabra*.

The traditional claim of the plant *C. tora* (Caesalpiniaceae) is used for the treatment of psoriasis, leprosy and other skin diseases. It has

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also been reported to exhibit a significant antioxidant activity (Manmohan Singha and Niraj Kansara, 2012; Gupta *et al*., 2013). *C. auriculata* belonging to the family Caesalpiniaceae, has traditionally been used to cure conjunctivitis, diabetes, and rheumatism. anti-inflammatory, antimicrobial, hepatoprotective, nephroprotective, anticancer, and antihyperlipidemic properties (Salma *et al*., 2020). Indian sarasaparilla, or *H. indicus* (Family: Asclepiadaceae), possesses antibacterial, anti-inflammatory, antioxidant, antidysen-teric, antipyretic, hepatoprotective, and antileprotic properties. In sweet drinks, these roots are utilized as a flavoring ingredient (Prathibha Devi Cherku *et al*., 2016; Sarita Das and Satpal Singh Bisht, 2013). The fruits of *T. chebula* belonging to the family Combretaceae, are abundant in fatty acids, polyphenols, flavanols, glycosides, triterpenoids, and hydrolysable tannins (32-34%). These phytochemicals support the high antioxidant and free radical scavenging activity, the wound healing and immunomodulatory activities, as well as the antibacterial, antifungal, antiviral, and anti-inflammatory activities (Mary Grace Jinukuti and Archana Giri, 2015; Aaron Mandeville and Ian Edwin Cock, 2018). Liquorice is a member of the Leguminosae family with the scientific name *G. glabra*. *G. glabra* an ayurvedic plant is frequently used in folk medicine and possess anti-inflammatory, antiviral, antibacterial, and anticarcinogenic properties (Leite *et al*., 2022; Nirmala and Selvaraj, 2011).

Hence, in the present study, herbal gel was formulated containing hydroalcoholic extract (70 %v/v) of the selected plants along with pharmaceutically acceptable excipients and evaluated for the management of acne.

2. Materials and Methods

The powdered samples of the selected plants for the proposed study were purchased from a commercial Siddha Medical Store Ansi Siddha and Ayurveda Store. Additional chemicals were acquired from Loba Chemie in Mumbai and HiMedia Laboratories Pvt. Ltd.

2.1 Extraction

The plant parts were dried at room temperature under the shade and powdered. About 250 g dried powder of each plant, *viz*., leaves of *C. tora,* flowers of *C. auriculata,* roots of *H. indicus,* seeds *of T. chebula,* and roots of *G. glabra* was macerated for three days in a ratio of 1:10 in 70% v/v ethanol, constantly agitated at 120 rpm using an orbital shaker. The extract was then filtered using gauze (0.1 mm 2 mesh) and Whatman filter paper (size 15 cm) (Whatman ® England), and these extracts were used for further studies (Harborne, 1998).

2.2 Preliminary phytochemical screening of extract

The prepared plant extracts were subjected to preliminary phytochemical screening for its phytoconstituents such as saponins, glycosides, phytosterols, tannins, flavonoids, carbohydrates, triterpenoids, polyphenol and alkaloids as per standard procedure (Khandelwal, 2004).

2.3 Formulation of herbal gel

The topical gels were prepared using hydroalcoholic (70 %v/v) extract of *C. tora,* flowers of *C. auriculata,* roots of *H. indicus,* seeds *of T. chebula,* and roots of *G. glabra* varying in concentration (Table 1). Propylene glycol, triethanolamine, Carbapol 940, and the necessary amount of water were used to prepare the gels in an amount adequate to produce 100 g of gel. Water required for these formulations was divided into two parts. In one part, accurate quantities of extracts was separately dissolved in 15 ml of water and to this calculated quantity of propylene glycol were added. In another part, Carbapol-940 was dissolved in 35 ml. Both of these solutions were mixed in a beaker and triethanolamine was added dropwise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel with required consistency. It was then stirred by using a propeller for 2 h at 500 rpm. After stirring, the prepared gel appeared to be homogeneous and devoid of any bubbles. The prepared gel was kept at room temperature for 24 h (Sri Agung Fitri Kusuma *et al*., 2018).

Table 1: Herbal gel formulation with varying herbal extract concentrations

S.No.	Ingredients	Amount taken per 100 gm (g)				
		F1	F2	F3	F4	Role of Ingredients
1.	*C. tora* leaves	0.05	0.1	0.2	0.3	Antikeratolytic and anti-inflammatory (Manmohan Singhal and Niraj Kansara, 2012)
2.	*C. auriculata* flower	0.05	0.1	0.2	0.3	Anti-inflammatory, antimicrobial and anti-oxidant (Salma *et al*., 2020)
3.	*H. indicus* root	0.05	0.1	0.2	0.3	Antioxidant, antibacterial and anti-inflammatory (Sarita Das and Satpal Singh Bisht, 2013)
4.	*T. chebula* fruit	0.05	0.1	0.2	0.3	Antioxidant, antibacterial and anti-inflammatory (Aaron Mandeville and Ian Edwin Cock, 2018)
5.	*G. glabra* root	0.05	0.1	0.2	0.3	Anti inflammatory and antibacterial (Nirmala and Selvaraj, 2011)
6.	Carbopol 940	1	1	1	1	Gelling agent
7.	Propylene glycol	2	2	2	2	Humectant/solvent
8.	Triethanolamine	0.5	0.5	0.5	0.5	Stabilizer or neutralizer
9.	Distilled water	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	

2.4 Physical evaluation of formulations

Physical parameters such as colour, viscosity, spreadability and extrudability were checked visually for the developed herbal gel (Supriya Agnihotri *et al*., 2016).

2.4.1 pH

A calibrated digital pH meter was used to monitor the pH of the aqueous solution (1%) of the formulation at a constant temperature.

2.4.2 Analysis of rheology

The viscosity of the formulated batches was measured with a Cone and Plate viscometer. In a procedure, a definite quantity of gel was added to a beaker covered with a thermostatic jacket. Spindle 7 was used to rotate the gel at a speed of 100 revolutions per minute.

2.4.3 Spreadability

Two sets of standard-sized glass slides were taken. Herbal gel was placed in between the two slides and sandwiched about the length of 60 mm. Removed the adhered excess gel on the surface of the glass slides and fixed to a stand without any disturbance. In the upper slide, 20 g weight was tied and noted the time taken for movement of the upper slide to the distance of 60 mm under the influence of weight. Meantime was calculated by repeating the experiment three times and the spreadability was calculated using the following equation:

$$\text{Spreadability} = (\text{Weight} \times \text{Length})/\text{Time}$$

2.4.4 Extrudability

Standard capped collapsible aluminium tubes were filled with the gel and sealed by crimping to the end. Weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 g was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The extruded gel's percentage was determined as >80% extrudability is good, >90% extrudability is excellent, and >70% extrudability is fair.

2.5 Antiacne activity

2.5.1 Microorganism

Sheep blood agar plates for *Propionibacterium acnes* and Muller Hinton agar for *Staphylococcus epidermidis* were the medium employed for the analysis. All media were purchased from HiMedia Laboratories Pvt. Limited. The test organism used in study was *Propionibacterium acnes* (MTCC No.1951) which was obtained from MTCC Chandigarh and *Staphylococcus epidermidis* (ATCC No. 12228) which was obtained from NCL, Pune, India.

2.5.2 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of a substance is its ability to prevent the growth of microorganisms. Mueller Hinton Broth for antibacterial screening was tested using the 96-well microtiter plate with lid (Wiegand *et al*., 2008). Briefly, 200 µl of the prepared gel in broth was introduced into the first wells in row A-B (in column 1). Columns 2-11 in rows A-B had 100 µl of broth alone while rows A-B in column 12 had 200 µl of broth. Twofold serial dilutions using a micropipette were done systematically down the columns 1-10 (from rows A-B). 100 µl was removed from the starting concentrations (columns 1-10 in rows A-B) and transferred to the next column with the 100 µl broth, properly mixed, and the procedure was repeated up to the last column (10) where the last 100 µl was discarded. This brings the final volume in all the test wells with the sample to 100 µl except the 12th column which had 200 µl of the broth that served as sterility control. An equal volume (100 µl) of the 1×10^6 CFU/ml bacterial (*S. Epidermidis*) inoculum was transferred into all the wells except the 12th column to give us the desired final inoculum load of 5×10^5 CFU/ml. Column 11 served as growth control (sample-free). Similarly, the sample was added in rows D-E with the aforesaid method of preparation for *P. acne*. The sample concentrations ranged from 1000 µg/ml to 2 µg/ml and the microtiter plate was incubated at 37°C for 24 h in a bacterial incubator for *S. epidermidis* and an anaerobic jar for *P. acne*. The lowest sample concentrations at which no visible bacterial growth were detected is known as the minimum inhibitory concentrations in the broth dilutions.

2.5.3 Antimicrobial assay of herbal antiacne gel

The well diffusion method was used to assess the antibacterial activity. Lyophilized culture of *P. acne* was revived in brain heart infusion broth at 37°C for 48 to 72 h under anaerobic conditions. *P. acne* inoculum was spread on the surface of sheep blood agar with the help of sterile swab stick. A well approximately 9 mm in diameter was bored on the surface of agar using a sterile cork borer. The samples (F1, F2, F3 and F4) were introduced into the well. The plates were then incubated at 37ºC for 48 to 72 h under anaerobic conditions in an anaerobic jar (Hi-Media) with gas pack and indicator tablet. (Anaerobic gas pack - a disposable oxygen absorbing and carbon dioxide generating agent for use in anaerobic jar was used to maintain and check the anaerobiosis. When an anaerobic tablet is introduced into a jar, its initial pink color indicates anaerobic conditions; if, however, it changes to a purplish-blue tint, it indicates aerobic conditions due to oxygen absorption).

S. epidermidis was inoculated in soybean casein digest medium (TSB) for 24 h at 37°C and adjusted to yield approximately 1.0×10^8 CFU/ml. *Staphylococcus epidermidis* inoculum was spread on the surface of Muller Hinton Agar with the help of sterile swab stick. The samples were then inoculated using same method performed above except that the plates were incubated at 37°C for 24 h under aerobic conditions. The control without plant extracts and 30 µg/ml of azithromycin were loaded wells that served as negative and positive controls, respectively. Using the antibiotic zone scale, the diameter of the zone of inhibition (mm) surrounding the well was measured in order to estimate the antimicrobial activity. (HiMedia, Mumbai, India) (Holder and Boyce, 1994).

3. Results

3.1 Extraction

The percentage yield values of hydroalcoholic (70% v/v) extract of *C. tora, C. auriculata,* H. *indicus, T. chebula,* and *G. glabra* were tabulated in Table 2.

Table 2: Percentage yield of the methanolic extracts of herbal drugs

Plant	Parts used	Percentage yield (w/w)	Colour
C. tora	**Leaves**	2.4	Dark green
C. auriculata	Flowers	1.8	Yellow
H. indicus	Roots	1.6	Brown
T. chebula	Fruits	3.5	Yellowish brown
G. glabra	Roots	2.8	Yellowish Brown

3.2 Qualitative phytochemical analysis

The qualitative phytochemical analysis of hydroalcoholic (70 %v/v) extract of *C. tora, C. auriculata, H. indicus, T. chebula,* and *G. glabra* indicate the presence of a number of secondary metabolites, including proteins, saponins, alkaloids, glycosides, flavonoids, tannins, steroids, and terpenoids (Table 3).

3.3 Formulation of herbal gel

The formulations were developed with hydroalcoholic (70% v/v) extract of *C. tora, C. auriculata, H. indicus, T. chebula,* and *G. glabra* using Carbopol 940 as a gelling agent. Because of their high user compliance, single-phase gel formulations for skin care are the preferred option. In such formulations, the active ingredient may be the secondary metabolites are uniformly distributed throughout a liquid leaving no apparent boundaries between the dispersed phytoconstituents and the liquid. Specifically, for acne vulgaris, the primary requisite is the formulation should spread easily and leave minimal residue or oiliness as it has been seen that oily skin type is more prone to acne problems. The developed formulation was light brown in color and formulation was glossy and translucent.

Table 3: Qualitative phytochemical analysis of the hydroalcoholic extract of *C. tora, C. auriculata, H. indicus, T. chebula* and *G. glabra*

S.No.	Phytoconstituents	*C. tora*	*C. auriculata*	*H. indicus*	*T. chebula*	*G. glabra*
1.	Alkaloids	+	+	+	-	-
2.	Saponins	+	+	-	-	+
3.	Carbohydrates	+	+	+	+	+
4.	Steroids	+	+	+	+	+
5.	Glycosides	+	+	+	+	+
6.	Triterpenoids	-	+	+	+	+
7.	Tannins	+	+	+	+	+
8.	Phenols	+	+	+	+	+
9.	Flavonoids	+	+	+	+	+

Note: + indicates presence, whereas - indicates absence.

3.4 Physical evaluation of herbal gel

As indicated in Table 4, the pH of the formulation was 6.7, which may be suitable for topical application without discomfort to avoid the risk of irritation upon application to the skin. The spreadability was 21.38-22.44 g.sm/sec, washable and viscosity was found in between 3660 ± 0.24 – 3964 ± 0.35 Cps. Extrudability of gel formulations was found to be excellent. This demonstrated that the technique might be used to generate topical dose formulations.

Table 4: Physical properties of herbal antiacne gel

Formulation	Parameters			
	pH	Viscosity (Cps)	Spreadability (gm.sm/sec)	Extrudability (%)
F1	6.7	3670 ± 0.38	22.16	91.82
F2	6.5	3660 ± 0.24	21.38	91.14
F3	6.6	3724 ± 0.31	21.84	89.36
F4	6.5	3964 ± 0.35	22.44	90.67

3.5 Minimum inhibition concentration: Microbroth dilution assay

The minimum inhibitory concentration of herbal gel against *P. acne* and *S. epidermidis* was tested on microbroth dilution method in a 96 well microtiter plate (Figures 13 and 14). The sample concentrations ranged from 2000 µg/ml to 4 µg/ml and the microtiter plate was incubated at 37°C for 24 h in a bacterial incubator for *S. aureus* and an anaerobic jar for *P. acne*. The results clearly revealed that there is no growth at the column 1-4 in all the rows A-C containing 1000-2 µg/ml. There was visible growth that can be seen from column 5-10 and 12 (Figure 1). Hence, the lowest concentration that inhibited the tested bacteria *P. acne* was found to be 125 µg/ml (Table 5).

The results clearly revealed that there is no growth at the column 1-5 in all the rows A-C containing 1000-2 µg/ml. There was visible growth that can be seen from column 6-10 and 12 (Figure 2). Hence, the lowest concentration that inhibited the tested bacteria *S. epidermidis* was found to be 63 µg/ml (Table 6).

Table 5: Minimum inhibitory concentration of *herbal gel* against *P. acne*

Concentrations at µg/ml												
Rows	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**
A	1000	500	250	**125**	63	31	16	8	4	2	0	0
B	1000	500	250	**125**	63	31	16	8	4	2	0	0

Arrow indicating the NO visible growth

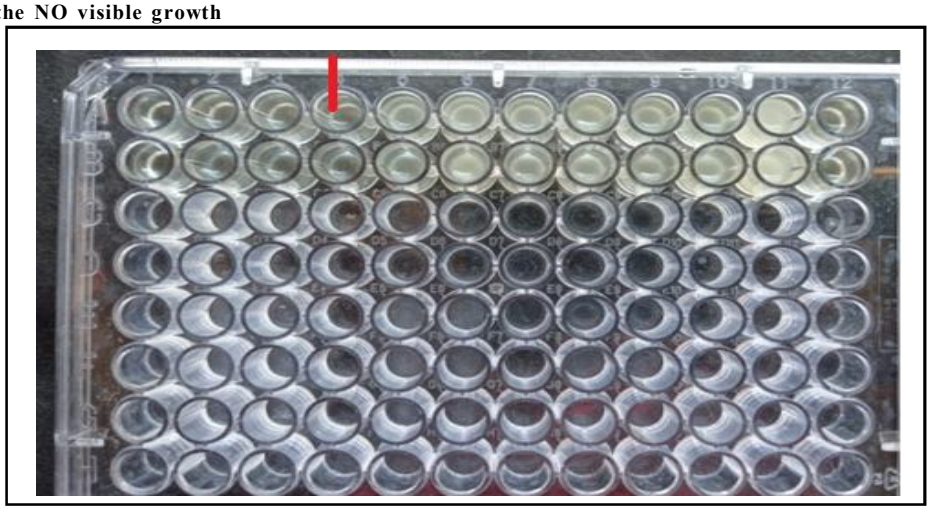

Figure 1: Minimum inhibitory concentrations activity of test sample against *P. acne*.
Column 1-10 (1000 µg/ml to 2 µg/ml); Column 11: Sample free control; Column 12: Sterility control; Rows A and B: Sample added wells.

Table 6: Minimum inhibitory concentration of herbal gel against *S. epidermidis*

Concentrations at µg/ml												
Rows	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**
A	1000	500	250	125	**63**	31	16	8	4	2	0	0
B	1000	500	250	125	**63**	31	16	8	4	2	0	0

Figure 2: Minimum inhibitory concentrations activity of test sample against *S. aureus*.
Column 1-10 (1000 µg/ml to 2 µg/ml); Column 11: Sample free control; Column 12: Sterility control; Rows A and B: Sample added wells.

3.6 Well diffusion method

Using the well diffusion method, the antibacterial activity of the herbal gel was evaluated against two acne-causing bacteria: *P. acne* and *S. epidermidis*. In the study, all the developed herbal antiacne gel formulations (F1-F4) except Control (F) showed inhibitory effect on *P. acne* and *S. epidermidis* (Table 7). The diameter of the inhibition zone was a parameter of a compound or substance that can still influence the bacteria. Among the tested formulation, F4 possessed highest zone of inhibition 24 mm against *P. acne* and 26 mm against *S. epidermidis* which was more effective than the standard azithromycin against *S. epidermidis*. (Figures 3 and 4).

Table 7: Antibacterial activity of test sample against *P. acne* and *S. epidermidis*

Name of the sample	Antibacterial activity (Zone of inhibition in mm)				
Formulated gel	F1	F2	F3	F4	Standard 30 µg (AZM)
P. acne	18	19	20	24	27
S. epidermidis	17	20	24	26	24

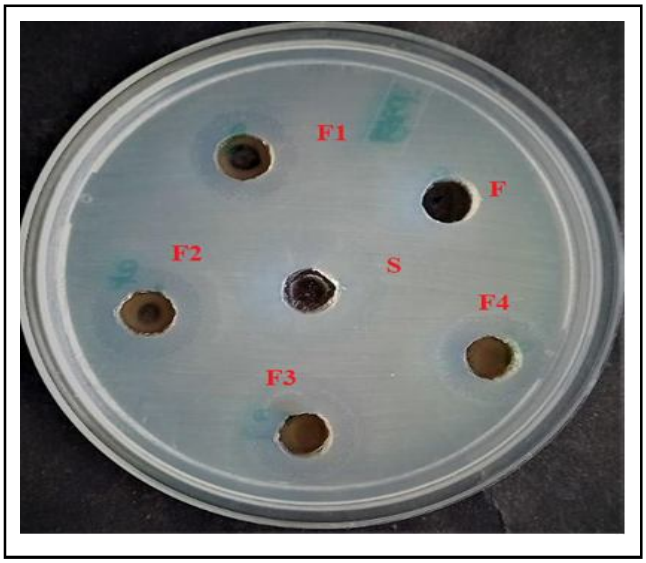

Figure 3: Antibacterial activity of herbal gel against *P. Acne*.

F: Control; F1-F4: Herbal gel formulation, S: Standard: Azithromycin (30 µg/ml).

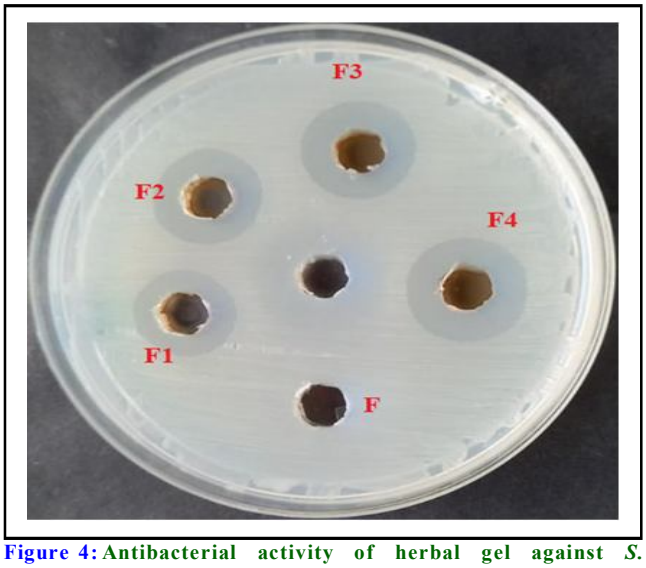

Figure 4: Antibacterial activity of herbal gel against *S. epidermidis*.

F: Control; F1-F4: Herbal gel formulation, S: Standard: Azithromycin (30 µg/ml).

4. Discussion

In the present study, herbal gel containing hydroalcoholic extract of selected plants with anti-keratolytic, anti-inflammatory, antimicrobial and antioxidant properties was developed for the management of acne. Water, ethanol, methanol and acetone are the most-used solvents for preparing extracts of high antioxidant activities, but when low toxicity or food applications are targeted, ethanol-water mixtures become the only acceptable solvent. Such mixtures were used to extract phenolics such as flavonoids, tannins, coumarins have been reported to exhibit antimicrobial, antioxidant, anti-inflammatory and skin protection from UV radiation activities (Dziao *et al*., 2016). Gels are becoming more and more popular. Compared to other semisolid preparations, including ointments, creams, pastes, *etc*., they can give controlled release and are more stable. Making gels can result in improved absorption, which increases medicinal drugs' bioavailability. Gels' long-term stability features open up possibilities for their beneficial application to patients (Chelladurai *et al*., 2023). In this investigation, Carbopol-940 was picked as a gelling operator in light of the fact that Carbopol was accounted for to have more gelling property with good plastic flow properties than another polymer and it is one of the excellent viscosity builders effective at low concentration and does not support microbial growth. Propylene glycol is a water miscible cosolvent for Carbopol®940 and acts as a preservative, humectant, plasticizer, or stabilizer in a variety of pharmaceutical formulations. Its penetration enhancement capability has been attributed to increased transdermal flux of many drugs (Allen, 1999; Weller, 2003; Panchangula, 2001). Extrudability of gel formulations was found to be excellent. The extrusion of the gel from the tube is an important during its application and in patient acceptance. Gels with high consistency may not extrude from tube whereas, low viscous gels may flow quickly and hence suitable consistency is required in order to extrude the gel from the tube (Satyabrata Bhanja *et al*., 2013).

The antibacterial activity of the herbal gel was evaluated against two acne-causing bacteria: *P. acne* and *S. epidermidis*. The formulation F4 possessed highest zone of inhibition of 24 mm against *P. acne* and 26 mm against *S. epidermidis* when compared with standard. The diameter of the inhibition zone was a parameter of a compound or substance that can still influence the bacteria. The greater diameter of the inhibition zone indicates that the bacteria were still sensitive to an antibacterial substance (Flanagan and Steck, 2017).

5. Conclusion

As natural medicines are thought to be safer and have less adverse effects than synthetic ones, they are more widely accepted. Herbs are safe, efficacious and multifunctional. Herbal formulations have growing demand in the world market. Establishing a herbal gel using hydroalcoholic extract of medicinal plants is a very good start.

From the entire study, it can be concluded that all four herbal anti-acne gel formulations showed antiacne activity against *P. acne* and *S. epidermidis,* however formulation F4 showed highest activity and per cent inhibition amongst all four formulated gels when compared with the standard azithromycin, a known antiacne agent. This could be because of the higher concentrations of herbal actives in Formulation F4 as against other formulations. This indicates that not just the right selection of actives but their concentrations in right quantities are also an essential controlling factor in achieving maximum activity.

It is also important for the formulated product to be stable for the stipulated time period and maintain its efficacy. This was determined by accelerated stress studies as per ICH guidelines. The Formulation F4 was stable throughout the stress studies carried out for three months to various stresses. The pH as well as water content was found to be well within limits at the end of the study. The efficacy of herbs used in acne treatment is due to their antibacterial activity and their influence on sebum activity, inflammation, and hyperkeratinization associated with acne.

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Conflict of interest

The author declares no conflicts of interest relevant to this article.

References

Aaron Mandeville and Ian Edwin Cock. (2018). *Terminalia chebula* Retz. fruit extracts inhibit bacterial triggers of some autoimmune diseases and potentiate the activity of Tetracycline. Indian J. Microbiol., **58**(4): 496-506.

Allen, L.V. (1999). The basics of compounding: Compounding gels. Int. J. Pharm. Compd., **3**(5):385-389.

Amal, A.; Ammar.; Hoda Salem; Shereen, A.; Eladawy. and Zinab I Abd Elsamad (2020). Development and clinical evaluation of topical hydroquinone niosomal gel formulation for the treatment of melasma. Int. J. Appl. Pharm., **12**(4):228-236.

Bialecka, A.; Mak, M.; Biedron, R.; Bobek, M.; Kasprowicz, A. and Marcinkiewicz, J. (2005). Different pro-inflammatory and immunogenic potentials of *Propionibacterium acnes* and *Staphylococcus*: Implications for chronic inflammatory acne. Arch. Immunol. Ther. Exp. **53**:79-85.

Chellathurai, B.J.; Anburose, R.; Alyami, M.H.; Sellappan, M.; Bayan, M.F.; Chandrasekaran, B.; Chidambaram, K. and Rahamathulla, M. (2023). Development of a polyherbal topical gel for the treatment of Acne. Gels., **9**:163.

Dessinioti, C.; Katsambas, A.D. (2010). The role of *Propioni-bacterium acnes* in acne pathogenesis: Facts and controversies. Clin. Dermatol., **28**:2-7.

Dzia³o, M.; Mierziak, J.; Korzun, U.; Preisner, M.; Szopa, J. and Kulma, A. (2016). The potential of plant phenolics in prevention and therapy of skin disorders. Int. J. Mol. Sci., **17**:160.

Fox, L.; Csongradi, C.; Aucamp, M.; Du Plessis, J. and Gerber, M. (2016). Treatment modalities for acne. Molecules. **21**:1063.

Gupta, V.; Rathore, D.S.; Niraj P. Kansara. and Badiger, A. M. (2013). *In vivo* antioxidant activity of topical cream of *Cassia tora* L. leaves extract. Dataset Paper in Pharmacology, pp:1-5.

Harborne. J.B. (1998). Methods of extraction and isolation. In: Phytochemical methods, (Chapman and Hall, London) pp:60-66.

Holder, I.A. and Boyce, S.T. (1994). Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns., **20**:426-429.

Khandelwal, K.R. (2004). Practical Pharmacognosy Techniques and Experiments, (Nirali Prakashan, Pune) pp:149-53.

Krakowski, A.C.; Stendardo, S. and Eichenfield, L.F. (2008). Practical considerations in acne treatment and the clinical impact of topical combination therapy. Pediatr. Dermatol., **25**:1-14.

Krautheim, A. and Gollnick, H.P.M. (2004). Acne: Topical treatment. Clin. Dermatol., **22**:398-407.

Lavers, I. (2014). Diagnosis and management of Acne vulgaris. Nurse Prescr., **12**:330-336

Leite, C.D.S.; Bonafé, G.A.; Carvalho Santos, J.; Martinez, C.A.R.; Ortega, M.M. and Ribeiro, M.L. (2022). The anti-inflammatory properties of licorice (*Glycyrrhiza glabra*)-derived Compounds in Intestinal Disorders. Int. J. Mol. Sci., **23**(8):4121.

Manmohan Singhal and Niraj Kansara. (2012). *Cassia tora* Linn. cream inhibits ultraviolet-B-induced psoriasis in rats. ISRN Dermatol., **2012**:346510.

Mary Grace Jinukuti and Archana Giri (2015). Anticancer activity of acetone and methanol extracts of *Terminalia chebula* Retz and *Withania somnifera* (Linn.) Dunal on HeLa cell line. Ann. Phytomed., **4**(2):88-92.

Nirmala, P. and Selvaraj, T. (2011). Anti-inflammatory and anti-bacterial activities of *Glycyrrhiza glabra* L. J. Agri. Tech., **7**(3):815-823.

Olutunmbi, Y.; Paley, K. and English, J.C. (2008). Adolescent female acne: Etiology and management. J. Pediatr. Adolesc. Gynecol., **21**:171-176.

Panchangula, R.; Salve, P.S.; Thomas, N.S.; Jain, A.K. and Ramarao, P. (2001). Transdermal delivery of naloxone: Effect of water, propylene glycol, ethanol and their binary combinations on permeation through rat skin. Int J Pharm., **219(1-2):**95-105.

Prathibha Devi Cherku, B.; Rama Devi, Veena Reddy and Syeda Fatima Manzelat (2016). Estimation of 2-hydroxy-4-methoxybenzaldehyde, lupeol and other unreported compounds in an elite ecotype of *Hemidesmus indicus* (L.) R. BR. Ann. Phytomed., **5**(1):51-58.

Salma, B.; Muthukumar, S.P.; Avinasha, S. and Manjula, S.N. (2020). Review on ethnobotany, phytochemistry, and pharmacological properties of *Cassia auriculata*. Pharm. Pharmacol. Int. J., **8**(2):106-111.

Sarita Das and Satpal Singh Bisht (2013). The bioactive and therapeutic potential of *Hemidesmus indicus* R. Br. (Indian Sarsaparilla) root. Phytotherapy Research, **27**(6):791-801.

Seidler, E.M. and Kimball, A.B. (2010). Meta-analysis comparing efficacy of benzoyl peroxide, clindamycin, benzoyl peroxide with salicylic acid, and combination benzoyl peroxide/clindamycin in ace. J. Am. Acad. Dermatol., **63**:52-62.

Sri Agung Fitri Kusuma, Marline Abdassah and Bobby Ellyas Valas. (2018). Formulation and evaluation of anti acne gel containing *Citrus aurantifolia* fruit juice using carbopol as gelling agent. Int. J. Appli. Pharm., **10**(4):147-152.

Supriya Agnihotri, Sharad Wakode and Anoop Agnihotri. (2016). Formulation and evaluation of herbal antiacne gel of *Myrica esculenta*. Asian J. Pharm. Clin. Res., **9**(4):358-361.

Weller, P.J. (2003). Handbook of Pharmaceutical Excipients, (American Pharmaceutical Association, Washington), pp:521-523.

Wiegand, K.; Hilpert and Hancock, R.E.W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols, **3**(2):163-175.

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